



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF ENERGY LEVEL ON OESTROUS BEHAVIOUR AND
FOLLICULAR DEVELOPMENT IN CATTLE IN MALAYSIA**

AZIZAH AMRI.

FP 2008 15

**EFFECTS OF ENERGY LEVEL ON OESTROUS BEHAVIOUR AND
FOLLICULAR DEVELOPMENT IN CATTLE IN MALAYSIA**

**By
AZIZAH BT AMRI**

**Thesis submitted, to Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master Science**

February 2008



DEDICATION

**WITH APPRECIATION AND RESPECT,
THIS THESIS IS DEDICATED**

TO

**MY HUSBAND AND CHILDREN: AHMAD NAZRI BIN ABDUL RAHMAN,
NURSHAZWANI, AHMAD SYAZWAN, AHMAD SYAZMI, AHMAD SYAZLI,
AHMAD SYAZNI, ALSO MY MOTHER PUAN SAMNAH BT SULBI**

WHO INSPIRED ME

AND

MADE IT

WORTHWHILE

ABSTRACT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EFFECTS OF ENERGY LEVEL ON OESTROUS BEHAVIOUR AND FOLLICULAR DEVELOPMENT IN CATTLE IN MALAYSIA

By

AZIZAH BT AMRI

February 2008

Chairman: Associate Professor Halimatun Yaakub, PhD

Faculty : Agriculture

The understanding of the development and growth of an ovulatory follicle within the ovary is important in order to improve the efficiency of breeding techniques such as artificial insemination (AI) and embryo transfer. The objectives of the study were to determine the effects of different levels of energy diets on characteristics of oestrous behaviour by visual observation, and follicular development in cows. The experiments were conducted to determine the oestrous behaviour by visual observation and the effects of dietary energy levels on follicular development in crossbred cows offered different levels and duration of energy diets. Thirty crossbred Kedah-Kelantan (KK) cows were divided into two groups, I) n=15; received grass with maintenance supplement and II) n=15; received grass with double maintenance supplement. Oestrous was initially synchronised using chloprostenol intra vaginal progesterone releasing device (CIDR-B[®]) containing 1.38 g progesterone for 7 days and administered 1 ml synthetic prostaglandin analogue 2 days before CIDR-B[®] removal. The cows were

observed for estrous behaviour continuously 24 hours daily for 72 hours immediately after CIDR-B[®] removal. The ovaries then were visualized using 7.5 MHz linear array transrectal transducer attached to a real-time, B mode portable ultrasound. Scanning was carried out from six hours at the beginning of estrous and repeated every six hours until ovulation. The day of first detection of a ≥ 4 mm follicle identified as a dominant follicle was taken as the first day of a follicular wave. During each ultrasound examination, a single blood sample was drawn and collected into 10 ml plain tubes for determination of progesterone concentration. A timed insemination was performed at 48 to 54 hrs after removal of CIDR-B[®] for all cows. Pregnancy diagnosis was performed using ultrasonography and rectal palpation techniques. In expressing oestrous behaviour characteristics, there were no differences between group of single maintenance (GSM) and group of double maintenance (GDM) ($p>0.05$) in the first cycle, while in the second cycle, characteristics of oestrous behaviour were clearly seen. The results obtained showed that approximately 33.3% of GSM and 40% GDM cows produced 2 waves of follicular development while 66.67% of GSM and 60% of GDM cows produce 3 waves of follicular development. Both GSM and GDM had equal number of cows producing 2 and 3 waves of follicular development. However, the number of waves of follicular development, length of estrous cycle, growth and regression rate of dominant follicle, and progesterone concentration between GSM and GDM were not significantly different ($p>0.05$). Therefore, the results from the experiment suggest that the higher and longer period of energy levels would not accelerate the estrous behaviour and increased the number of animals pregnant. From the study, it

was concluded that cows offered double maintenance levels of energy diet was not significantly different from cows offered single maintenance levels of energy diet in terms of exhibit oestrous behaviour, follicular development and number of animal pregnant ($P>0.05$).

ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN ARAS TENAGA KE ATAS PEMBENTUKAN FOLIKEL PADA LEMBU

Oleh

AZIZAH BT AMRI

Februari 2008

Pengerusi: Profesor Madya Halimatun Yaakub, PhD

Fakulti: Pertanian

Pemahaman mengenai pembentukan dan pembesaran folikel pengovul pada ovari adalah penting untuk memperbaiki kecekapan teknik pembiakan seperti peranakan beradas (AI) dan pemindahan embrio. Objektif pembelajaran adalah untuk menentukan kesan aras tenaga diet yang berbeza ke atas perlakuan estrus melalui pemerhatian dan pembentukan folikel pada lembu betina. Eksperimen telah dijalankan untuk menentukan tingkahlaku estrus melalui pemerhatian secara visual dan kesan aras tenaga ke atas pembentukan folikel lembu betina baka silang. Tiga puluh ekor lembu betina Kedah-Kelantan (KK) baka silang dibahagikan kepada dua kumpulan, I) n=15, mendapat rumput dengan makanan tambahan penyelenggaraan dan II) n=15, mendapat rumput dengan makanan tambahan 2 kali ganda penyelenggaraan. Estrus diselaraskan dengan menggunakan CIDR-B[®] yang mengandungi 1.38 g progesterone selama 7 hari dan 1 ml analog prostaglandin sintetik diberi 2 hari sebelum CIDR-B[®] dikeluarkan dari vagina.

Perlakuan estrus dijalankan melalui pemerhatian 24 jam sehari secara berterusan selama 72 jam bermula dari CIDR-B[®] dikeluarkan. Kedua-dua ovari digambarkan menggunakan 7.5 MHz 'linear array transrectal transducer' yang dilekapkan bersama ultrasound mudahalih 'B-mode real-time'. Penemuan pertama folikel bersaiz ≥ 4 mm dikenalpasti sebagai folikel dominan dan diambil sebagai hari pertama gelombang bermula. Sampel darah dipungut dan disimpan ke dalam tiub kosong untuk penganalisaan kepekatan progesterone. Penetapan masa Inseminasi dijalankan pada 48 dan 54 jam setelah CIDR-B[®] dikeluarkan dari kesemua lembu betina. Diagnosa kebuntingan dijalankan dengan menggunakan kaedah ultrasnografi dan teknik palpasi rekta. Keputusan menunjukkan tidak ada perbezaan bererti diantara GSM dan GDM ($p>0.05$) pada ciri perlakuan estrus pusingan pertama, tetapi boleh dilihat secara jelas pada perlakuan estrus pusingan kedua. Keputusan yang diperolehi menunjukkan 33.3% dan 40% daripada lembu betina dari GSM dan GDM masing-masing sekurang-kurangnya menghasilkan 2 gelombang folikular manakala 66.7% dan 60% daripada GSM dan GDM masing-masing terdiri daripada 3 gelombang folikular. Kedua-dua GSM dan GDM mempunyai bilangan lembu betina yang sama terdiri dari 2 dan 3 gelombang pembentukan folikular. Walaubagaimanapun, bilangan gelombang pembentukan folikular, jangkamasa pusingan estrus, kadar pertumbuhan dan pengecutan folikel dominant, dan aras kepekatan progesterone di antara GSM dan GDM tidak ada mempunyai perbezaan yang bererti ($P>0.05$). Oleh yang demikian keputusan dari eksperimen mencadangkan kandungan yang tinggi dalam jangkamasa yang lama tidak mempercepatkan perlakuan estrus dan mempertingkatkan bilangan ternakan

yang bunting ($P>0.05$). Dari pembelajaran ini, kesimpulan yang diperolehi adalah tiada hubungan yang bererti diperolehi di antara lembu betina yang diberi dua kali aras tenaga diet pengambilan makanan penyelenggaraan dengan lembu betina yang diberi sekali aras tenaga diet makanan penyelenggaraan berkaitan dengan perlakuan estrus, pembentukan folikel dan bilangan ternakan yang bunting ($P>0.05$).

ACKNOWLEDGMENTS

I would like to express my sincere gratitude and appreciation to my supervisor Assoc. Prof. Dr. Halimatun Yaakub for her continuous encouragement, invaluable guidance, advice, tolerance, supervision, and support throughout the course of this study.

I wish to express my gratitude to my co-supervisor Assoc. Prof. Dr. Sabrina Sukardi and Prof. Dr. Abd. Razak Allimon from Department of Biomedical, Faculty of Medicine and Health Science and Department of Animal Science, Faculty of Agriculture, Universiti Putra, respectively, for their advice and insightful suggestions that help towards the completion of this study.

Sincere thanks also to all MARDI's staff in Kluang, Johore, En. Ajis Hasan, En. Mashodi Surip, En. Awis, En. Mat Sallehhudin Abd. Aziz and En. Ali Ahmad, for their kindness in allowing me to pursue pertinent work and use of their farm facilities for this study.

I wish to thank my beloved husband, En. Ahmad Nazri bin Abdul Rahman for his patience and understanding in giving permission and understanding throughout of my study, and all my colleagues who have closely helped me in the laboratory work, computer work, advice and moral support in completing the thesis.

I certify that an Examination Committee has met on 18 February 2008 to conduct the final examination of Azizah binti Amri on her Master of Science thesis entitled "Effects of Energy Level on Oestrus Behaviour and Follicular Development in Cattle in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.


Members of the Examination Committee were as follows:

Ismail Idris, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Jothi Malar Panandan, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Shanti Ganabadi, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Mahanem Mat Noor, PhD
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)


HASANA MOHD. GHAZALI, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

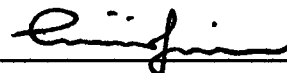
Date: 22 July 2008

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Halimatun Yaakub, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Abdul Razak Alimon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Sabrina Sukardi, PhD
Associate Professor
Faculty of Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 August 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



AZIZAH BT AMRI

Date: 15 July 2008

TABLE OF CONTENTS

	Page
DEDICATION	i
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
CERTIFICATION	ix
APPROVAL	x
DECLARATION	xi
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF PLATES	xvi
LIST OF ABBREVIATIONS	xvii

CHAPTER		Page
1	INTRODUCTION	1
2	LITERATURE REVIEW	
	2.1 Oestrous behaviour and detection	7
	2.2 Characteristic signs of oestrous behaviour	9
	2.3 Oestrous synchronization	10
	2.4 Factors influencing the oestrous signs and behaviour.	12
	2.5 Factors influencing the oestrous cycle	13
	2.5.1 Hormonal influence	13
	2.5.2 Follicular development	13
	2.5.3 Nutritional influence	15
	2.6 Factors influencing the follicular development	15
	2.6.1 Growth hormone and insulin	15
	2.6.2 Follicle Stimulating Hormone (FSH)	16
	2.6.3 Luteinizing Hormone (LH)	16
	2.6 Nutritional factor influences on oestrous behaviour and follicular development	17
	2.7.1 Energy	19
	2.7.2 Protein	19
	2.8 Pattern of follicular wave.	19
	2.9 Follicular wave dynamics in cattle	21
3	MATERIALS AND METHOD	
	3.1 Animal management and treatment	22
	3.2. Feed	23
	3.3 Synchronisation of oestrus	27
	3.4 Oestrus behaviour observation	27
	3.5 Scanning of the ovaries	30
	3.6 Interovulatory Interval Categories	33



	3.7 Artificial insemination and pregnancy diagnosis	34
	3.8 Blood collection	34
	3.9 Progesterone (P ₄) assays	37
	3.10 Statistical analyses	38
4	RESULTS	40
5	DISCUSSION	55
6	CONCLUSION	64
7	REFERENCES	66
	APPENDICES	77
	BIODATA OF THE STUDENT	100
	LIST OF PUBLICATION	101
	SEMINARS/WORKSHOPS/CONFERENCE ATTENDED	103
	AWARDS OBTAINED	104
	RESEARCH GRANTS	104

LIST OF TABLES

Table	Title	Page
3.1	The proximate analysis of the commercial pellet feed and grass based on the dry matter (%)	25
4.1	The oestrus sign observed at the first and the second oestrous cycle of cows fed on diet with different energy levels (hrs \pm se).	41
4.2	The number and percentage of cows with and without oestrus signs fed on different energy level.	43
4.3	The number and percentage of cow with 2 or 3 waves observed after synchronisation.	44
4.4	Effect of energy levels on the follicular wave development in KK crossbred cows post synchronisation.	44
4.5	Comparison of 2 and 3 waves of KK crossbred cow follicular wave development (mean \pm se) post synchronisation supplied with different energy level.	47
4.6.	Mean follicular diameter and hormonal concentrations within the waves of follicular development during 2-wave and 3-wave of follicular development.	48
4.7	Effect of different level of energy diets (single maintenance: GSM; double maintenance: GDM) on the number and percentage of animal pregnant and not pregnant diagnosed using ultrasonography and rectal palpation techniques on Day 30 and Day 90 post AI, respectively.	54

LIST OF FIGURES

Figure	Title	Page
3.1	Experimental protocols: synchronisation, day of dietary treatment started, oestrus, AI (after the 3 rd normal oestrous) six and twelve hours post standing oestrous) and pregnancy diagnosis post AI (Day 30: ultrasonography and Day 90: rectal palpation techniques).	28

LIST OF PLATES

Plate	Title	Page
3.1	Cows were released for grazing at 16.00 to 17.00 hours in the evening every day.	26
3.2	Cows were kept individual pens size 9' x 6' during pellet feeding time.	26
3.3	Mounting activity of cows on the pen sizes 9'x 6' for 72 hours period	29
3.4	The white thick fluid-like (mucus) was observed on tips of the vagina	29
3.5	Transducer was inserted through rectum to visualize the ovary stroma.	31
3.6	Transducer and ultrasound SSD-500 used for Follicular mapping.	31
3.7	Image of the follicles obtained from ultrasound.	33
3.8	Blood was collected through the jugular vein using 18G needle into a serum tube.	36
3.9	Blood was maintained in the container with surrounded ice pack to maintain the temperature of 5-8°C.	36

ABBREVIATIONS

The following abbreviations are used in the thesis with or without definition.

AI	Artificial insemination
ANOVA	Analysis of variance
ARC	American Research Council
B/Bo	Percent bound
BST	Bovine somatotrophin
BW	Body weight
cAMP	Cyclic AMP
CL	Corpus luteum
CF	Crude fibre
CP	Crude protein
CPM	Count Per minute
CIDR/CIDR-B®	Chloprostenol intravaginal progesterone releasing device
d	Day
DFs	Dominant follicles
DM	Dry matter
EE	Ether extract
E/P	Estradiol prostaglandin
F	Fasting
FSH	Follicle Stimulating Hormone
GE	Gross energy
GSM	Single maintenance group
GDM	Double maintenance group

h	Hour
IBHK	National Institute of Biotechnology
IGF	Insulin-like growth factors
IQC	International Quality Control
KK	Kedah-Kelantan
Kg	Kilogram
LH	Luteinizing Hormone
M	Maintenance
MARDI	Malaysia Agriculture Research and Deelpment Institute
MINT	Institute for Nuclear Technology Research
MGA	Melengesterol acetate
MJME	Mega Joule Metalbolisme energy
MJ	Mega Joule
mL	Milliliter
<i>mL</i>	Microliter
<i>mg</i>	Microgram
NEM	Net energy for maintenance
ng/mL	Nanogram per milliter
NRC	National Research Council
ODB	Oestradiol benzoate
OPF	Oil Palm Fronds
PG	Prostaglandin analogue
P₄	Progesterone
PKC	Palm Kennel Cake
POME	Palm mill Effluent

W	Weight
MHz	Mega hertz
PD	Pregnancy diagnosis
SBM	Soyabean meal
RIA	Radioimmunoassays
^{125}I	Deionized iodine 125
TC	Total count
SPSS	Statistical analysis for Social Science
IVP4	Intravaginal progesterone releasing inserts
SAG	Sexually active group

CHAPTER 1

INTRODUCTION

Oestrus is the manifestation of sexual receptivity in the female mammal. Oestrous signs or behaviour is exhibited just before ovulation in cattle and is relatively short (2 to 3 % of the oestrous cycle) and usually last for 18-19 hours (ranging from 14 to 22 hours) (Hafez *et al.*, 2000). Each oestrous cycle in cattle is divided into four stages, pro-oestrus, oestrus, metoestrus and dioestrus.

Oestrus, the animal will show signs indicating the receptivity to mate. The female will attempt to mount, lick, show nervous signs and stand to be mounted by others. The vagina will become reddened with presence of mucous discharge (watery to thick) when it is close to ovulation (Roelofs *et al.*, 2005a). Various methods are being used to detect oestrous signs such as tail-paint and chalk, intravaginal and vulvar signs, electrical impedance, vasectomized bulls or continuous visual observation and radiotelemetry (Stevenson *et al.*, 1996; Xu *et al.*, 1998; Cavailieri *et al.*, 2003b). The information of when the cow showed the onset of oestrus renders possibility to estimate the optimum time to conduct artificial insemination. In addition, it is also important to detect the duration of oestrus in cattle in order to reduce the cost when practicing artificial insemination (AI) technique (Cobert *et al.*, 1999). Therefore, the observation of oestrous signs or behaviour is beneficial

in order to conduct the AI at the correct time to increase pregnancy rates (Colazo *et al.*, 2004a, b).

Synchronisation of oestrus in cattle can facilitate the use of AI by reducing the time needed for detection of oestrus compared to cattle entering oestrus spontaneously. After progestin released subcutaneous implants or intravaginal progesterone releasing inserts (IVP4) were used to synchronize oestrus, up to 85% of cattle can be induced to enter oestrus between 36 and 60 hour after the end of treatment (Diskin *et al.*, 2002).

Nutrition has a key role in regulating reproductive processes in cattle. The effects of acute nutritional restriction in cattle are not known. Nutrition is an important element in altering the development diameter of follicles apart from its importance for development of dominant follicle size. It has been reported that the diameter size of dominant follicles decreased when feed is restricted to the animals (Murphy, 1991; Bergfeld, 1994; Rhodes, 1995). Apart from this Mackey *et al.* (1997a, 1999, 2000) concluded that an acute restriction in energy intake 3 to 6 days before ovulation decreased the growth rate and maximum size of the preovulatory dominant follicle. This subsequently resulted in fewer follicles emerging in the second follicular wave.

The main energy substrates used by ruminants are volatile fatty acids (acetate, butyrate and propionate) produced in the rumen. The concentration of volatile fatty acids profiles can be different due to differences in the rumen degradation of roughages (Archimede *et al.*, 1996). The highly degradable

feed in the rumen, especially in excessive feeding with protein sources can reduce fertility in dairy cattle (Lanyasunya *et al.*, 2005). Butlers *et al.* (1996) indicated that high milk or plasma urea concentrations are clearly associated with decreased fertility in dairy cows. Alternatively, imbalances in the relative availability of protein and energy may affect efficiency of metabolism and energy status. In cattle, the relationship between dietary intake and progesterone (P_4) concentration in blood is controversial (Spitzer, 1986, 1978; McCann *et al.*, 1986). Lower P_4 could lead to prolonged follicular growth associated with lower fertility probably due to the decrease in oocyte quality (Mihm *et al.*, 1994, 2003).

The ability of animals to conceive also depends on the right time to introduce the semen into the vagina of the female. Therefore, the oestrous detection, time and technique of AI, and the quality of semen are very important. It is known that insemination between 12-24 hours after the beginning of standing oestrous is the best time to ensure that sperm arrive at the site of fertilization before ovulation (Peters *et al.*, 1995). Therefore, with the information gathered, researchers were able to establish the optimum time to conduct artificial insemination.

Recently, research in cattle reproduction has focused on the development and growth of an ovulatory follicle within the ovary. In order to improve the efficiency of breeding techniques such as AI and embryo transfer, the knowledge regarding the development and control of the ovarian follicular development is required.

During the oestrous cycle, a number of follicles are selected to complete differentiation and ovulation. Follicular development involves three stages, which are recruitment, selection and dominance. The process whereby a cohort of follicles is formed from a larger number of smaller follicles is termed as recruitment. The single follicles in each cohort will be selected and continue to grow to become dominant and suppresses the growth of other follicles in the cohort (Rajakoski, 1960; Adams, 1994, 1995; Pierson and Ginther, 1987, 1988b).

The study of follicular dynamics can be obtained by visualizing bovine ovaries using transrectal ultrasonography (Pieterse *et al.*, 1991; Pierson and Adams, 1988b; 1999; Vassena *et al.*, 2003; Vinales *et al.*, 2004). It is performed in order to determine large variations in the turnover of follicles and timing of ovulation. Transrectal ultrasonic imaging of ovarian follicles reveals that most oestrous cycles in cows have two or three follicular waves. Follicular growth is a process where a cohort of follicles began to mature and grow under the influence of sufficient pituitary gonadotropic stimulation to permit progression towards ovulation (Ireland *et al.*, 1983a, 1983b, 1987; Lucy *et al.*, 1992; Machatkova *et al.*, 2000). The follicular waves are first detected on 4-5 mm follicles and can be recognized in a series of follicular development as the growing, static and regressing phases. The period from emergence of a wave to apparent cessation of progressive growth is defined as the growth phase. Plateau phase is the period from cessation of growth to the apparent onset of progressive decreases in diameter and regressive phase can be defined as the period after the end of the plateau phase (Rajakoski, 1960). The